EXHIBIT 601.2

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Correlation of Antemortem and Postmortem Digoxin Levels

The dynamics of digoxin metabolism have been well studied since the introduction of sensitive radioimmunoassay procedures capable of detecting low biologic levels of this compound [*1*]. The value of monitoring digoxin therapy through serum levels has been widely accepted, and toxic effects are frequently observed when the serum level of adult individuals exceeds 2 ng/ml [*2,3*]. Beller et al [*4*] reported a twofold increase in mortality among hospitalized patients having digitalis toxicity.

Only a few authors have reported on postmortem digoxin levels. Several of these have assumed that serum levels obtained from intracavitary myocardial autopsy specimens accurately reflect antemortem levels at the time of death [*5-7*]. Others have noted possible difficulties in interpreting postmortem values. Iisalo and Nuutila [*8*] in 1973 pointed out discrepancies between antemortem and postmortem serum digoxin levels in three cases and attributed this to "accumulated absorption," presumably prior to death. Karjalainen et al [*9*] in 1974 stated "the postmortem concentrations of blood digoxin are higher than those measured during life" but gave no explanatory or supporting data. Selesky et al [*10*] also thought that some of the elevated postmortem digoxin values seen in their series may have been due to the interval between death and sampling, although in the one case in which an antemortem specimen was analyzed there was no difference in value between the serum obtained before death and that taken at autopsy.

Holt and Benstead [*11*] in 1975 reported another problem with the interpretation of postmortem digoxin values. They demonstrated that digoxin levels on serum taken from heart blood at autopsy were consistently higher than levels on samples from the femoral veins, with the difference as great at 137% in their series. Dickson and Blazey [*12*] in 1977 stated they found a similar heart to venous blood ratio in one case and pointed out that most previous reports had not indicated the site from which the serum samples were obtained.

The purpose of the present study is fourfold: to determine the discrepancies that exist between antemortem and postmortem digoxin levels, to learn if such differences can be related to the postmortem interval, to substantiate variation in postmortem blood values between samples taken from different sites, and finally to establish the most accurate way of estimating digoxin toxicity from postmortem specimens.

Materials and Methods

Twenty-seven autopsy cases from two county hospitals were studied. Postmortem samples from the left ventricular cavitary blood and vitreous humor were obtained in all cases. Sub-

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clavian and femoral venous blood samples were obtained in 24 and 11 cases, respectively. All patients were receiving therapeutic doses of digoxin by various routes of administration for at least one week prior to death. No cases of suicidal or acute accidental poisoning were included. Antemortem serum samples were available for analysis in all cases, and the last digoxin dose was administered a minimum of 4 h before an antemortem sample was obtained. The blood urea nitrogen and interval between death and postmortem sampling (postmortem interval) were recorded.

Samples were centrifuged immediately, and the serum was refrigerated at 4 °C. Assays were performed within 72 h. In one hospital (St. Paul-Ramsey) assays were performed with material from Corning Medical Diagnostics, while in the second institution (HCMC) the materials used were from New England Nuclear. The procedure in either case was based on the principles of Smith et al [*1*] and involved the competitive binding of ^{125}I-labeled digoxin and unlabeled digoxin (present in the serum or vitreous sample) with a specific rabbit anti-digoxin antibody. The digoxin bound to antibody was separated by absorbing out the unbound digoxin (both labeled and unlabeled) with charcoal. In the Corning procedure this step was omitted because the antibody was precoated to glass beads. The bound fraction was counted with a gamma scintillation counter, and the percentage of bound ^{125}I-labeled digoxin was calculated. The digoxin level in the sample was determined from a standard curve, constructed daily by using five standards. Corning and New England Nuclear standards ranged from 0 to 5.0 ng/ml and 0.5 to 8 ng/ml, respectively. There was no difficulty in performing the postmortem tests with either procedure. The results were both internally consistent and showed good correlation between the two institutions when analyzed statistically.

The time from antemortem sampling to death ranged from 1 to 48 h, with a mean of 7.7 h. It was therefore necessary to correct for continued metabolism of the drug during the interval between the antemortem sample and death by determining the drug half-life in each case. Approximately 90% of digoxin was excreted unchanged by the kidney, and the half-life was therefore related to renal function. Each patient had a blood urea nitrogen (BUN) test performed within one day of death, and drug half-lives were determined by using data published by Jelliffe [*13*]. The immediate antemortem digoxin level was then calculated by using the formula

$$\ln N_t = \ln N_0 - \ln 2(t/T\tfrac{1}{2})$$

where

N_t = serum digoxin level at time of death,
N_0 = serum digoxin level at time of antemortem sampling,
t = time interval between drawing of antemortem sample and death, and
$T\frac{1}{2}$ = digoxin half-life based on BUN [*13*].

Results

Postmortem intervals ranged from 1.0 to 22.4 h, with a mean of 10.8 h. Compared to antemortem levels, average postmortem serum digoxin levels were significantly higher ($P < 0.001$) in samples taken from the heart, subclavian vein, and femoral vein (Table 1). Postmortem cardiac serum levels exceeded antemortem levels in all 26 cases where the postmortem interval was greater than an hour, and in no case did the postmortem level fall below the antemortem values on samples from heart or subclavian vein. In contrast, 2 of the 11 cases in which femoral samples were drawn had lower postmortem serum values than the corresponding antemortem levels (Cases 1 and 26). The mean of postmortem to antemortem ratios was 1.96 for heart, 1.63 for subclavian, and 1.42 for femoral samples. Vitreous levels were somewhat more variable, with 23 falling below, 3 exceeding, and 1 equaling antemortem levels. The mean ratio of vitreous to antemortem values was 0.71.

TABLE 1—*Comparison of antemortem and postmortem serum digoxin levels.*

Case	BUN,[a] mg/dl	Serum Digoxin Levels, ng/ml[b]					
		Antemortem		Postmortem			
		At Time of Sampling	Calculated for Time of Death	Heart	Subclavian	Femoral	Vitreous
1	24	17.3	15.8	25.8	18.2	15.2	10.4
2	27	8.2	8.0	9.8	9.2	...	2.4
3	122	6.6	6.6	8.8	8.4	...	4.2
4	59	5.1	4.8	9.4	9.8	7.5	3.8
5	44	4.5	4.0	7.8	7.5	5.6	3.8
6	70	4.0	3.8	7.0	7.9	...	2.1
7	100	3.5	3.4	5.7	5.6	...	2.1
8	87	3.3	3.2	4.2	3.8	...	1.5
9	94	2.7	2.7	2.7	3.0	...	1.4
10	41	3.0	2.4	5.2	4.4	3.1	2.0
11	14	2.7	2.2	4.5	4.2	3.5	2.1
12	150	2.1	2.0	5.4	3.2	...	2.4
13	30	2.3	2.0	3.5	...	...	1.5
14	20	2.0	1.9	4.7	3.1	2.9	1.4
15	22	1.8	1.8	5.0	...	...	1.8
16	78	1.9	1.6	2.2	2.0	...	0.6
17	28	1.9	1.6	4.8	...	...	1.3
18	75	1.7	1.6	2.0	1.8	...	1.1
19	56	1.6	1.4	3.5	2.6	...	0.5
20	90	1.5	1.4	3.2	2.1	...	0.6
21	16	1.5	1.4	2.5	2.2	2.0	0.7
22	40	1.4	1.3	1.7	1.3	...	0.7
23	14	1.2	1.2	2.3	1.6	1.3	0.4
24	22	1.4	1.2	2.7	2.3	2.2	1.0
25	56	1.8	1.0	2.9	2.5	2.3	1.1
26	22	0.6	0.6	1.2	0.9	0.4	0.4
27	20	0.5	0.3	0.8	0.9	...	0.5

[a] All urea nitrogen values were obtained from blood specimens drawn less than 24 h before death.
[b] All cases in which a femoral specimen was obtained were from one institution (HCMC) and the materials for assay were supplied by New England Nuclear. The remaining tests were performed in the second hospital with assay material from Corning Medical Diagnostics.

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No useful correlation could be made between postmortem interval and either the absolute or relative change in postmortem samples, regardless of the site of sampling (Table 2). Case 9 had the shortest postmortem interval and the least amount of change; however, this trend did not hold true for a major portion of the cases. Case 17 showed the greatest relative increase in heart level with a postmortem interval in the mid-range, while Case 2, with a much longer postmortem interval, showed the second lowest change of the series.

If a diagnosis of digoxin toxicity were to be made on the basis of serum levels equal to or greater than 2 ng/ml, 48% of patients in this series would have been so diagnosed with the immediate antemortem value. With the postmortem heart samples, the figure would rise to 89%. Similarly, subclavian and femoral samples also lack specificity for digoxin toxicity. However, a postmortem serum level below 2 ng/ml does appear to be strong evidence against toxic levels in the antemortem stage.

Although the vitreous concentrations also differed markedly from true antemortem concentrations, they were more accurate indicators of toxicity. Ten patients (37%) had vitreous levels equaling or exceeding 2.0 ng/ml. Each of these ten cases also had a toxic antemortem serum level. Only three cases of possible toxicity (based on elevated antemortem levels) were not detected by vitreous determinations.

TABLE 2—*Ratio of postmortem to antemortem digoxin levels and relationship to postmortem interval (PMI).*

Case	PMI, h	Postmortem to Antemortem Ratio			
		Heart	Subclavian	Femoral	Vitreous
1	17.0	1.63	1.15	0.96	0.67
2	15.2	1.22	1.15	...	0.30
3	5.7	1.33	1.27	...	0.64
4	19.0	1.96	2.04	1.56	0.79
5	13.0	1.95	1.88	1.40	0.95
6	21.0	1.84	2.08	...	0.55
7	20.9	1.68	1.65	...	0.62
8	4.0	1.31	1.18	...	0.47
9	1.0	1.00	1.11	...	0.52
10	13.5	2.17	1.83	1.29	0.83
11	10.8	2.04	1.91	1.59	0.95
12	16.3	2.70	1.60	...	1.20
13	6.5	1.75	...	...	0.75
14	16.2	2.47	1.63	1.52	0.74
15	14.0	2.78	...	...	1.00
16	22.4	1.38	1.25	...	0.38
17	11.3	3.00	...	...	0.81
18	4.4	1.25	1.12	...	0.69
19	15.0	2.50	1.86	...	0.36
20	10.8	2.28	1.50	...	0.43
21	3.5	1.78	1.57	1.43	0.50
22	2.5	1.31	1.00	...	0.54
23	3.8	1.92	1.33	1.08	0.33
24	3.4	2.24	1.42	1.83	0.83
25	3.0	2.90	2.50	2.30	1.10
26	16.0	2.00	1.50	0.67	0.67
27	6.8	2.67	3.00	...	1.67
Mean	10.6 ± 6.26	1.96 ± 0.56	1.63 ± 0.48	1.42 ± 0.44	0.71 ± 0.30
Correlation with PMI, *r*	...	0.15	0.14	0.48	−0.06

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Discussion

It is clear from this investigation that postmortem digoxin levels taken from cardiac blood, venous blood, or vitreous humor do not mirror the antemortem levels. Substantial increases in serum levels occur following death, irrespective of the source of the sample. It seems probable that a new drug equilibrium between blood and tissue is established after death. Several investigators have found various tissue levels to exceed blood levels, with the highest concentrations occurring in the heart and kidney [*8,9,14*]. The ratio of myocardial to serum concentrations is approximately 30:1. With cell death and subsequent loss of membrane integrity, digoxin must diffuse from tissue into the adjacent circulatory compartment. As a consequence it is possible to falsely diagnose digoxin toxicity from postmortem serum specimens no matter what the source of the samples, but a postmortem serum level below 2 ng/ml will exclude the presence of toxic levels in the antemortem state.

Conversely, vitreous levels are usually below the true antemortem values. The vitreous compartment appears to be less permeable to compounds in the circulation. DiMaio et al [*6*] have suggested that vitreous to serum ratios less than one reflect rising blood levels at the time of death and those greater than one reflect falling levels. Our data do not support such a concept. All patients died between 6 and 120 h after their last dose. Since the time required for equilibration with blood and myocardium is usually less than 4 h after oral administration, blood levels should have been falling in every case. It is possible that in most individuals vitreous levels never equilibrate with blood. However, this series does establish that significantly elevated vitreous levels correspond with toxic antemortem serum levels.

A larger series correlating true antemortem with postmortem concentrations might establish a serum or vitreous threshold concentration that most accurately reflects the clinical situation. In the absence of such a threshold concentration for guidance, we think that a combination of venous serum and vitreous humor values provide the most useful information. Femoral samples appear preferable to subclavian.

A striking finding of this study is that 14 of 27, or 52%, of the patients had toxic levels at the time the antemortem samples were drawn, most of which were obtained for electrolyte or cardiac enzyme determination. Digoxin toxicity was thought to be the cause of death in Case 1 and may well have been a contributory factor in the deaths of Cases 2, 3, and 4. We have found this type of retrospective analysis useful and strongly recommend saving serum for five to seven days in the laboratory. The cardiac glycoside dosage is frequently not adjusted for acute renal failure or renal hypoperfusion commonly seen in severely ill patients.

This investigation also raises suspicion regarding many studies in postmortem toxicology. If indeed a new blood-tissue equilibrium is established with digoxin after death, a similar situation may exist for many compounds studied during autopsy. Gee [*15*] has already reported varying barbiturate levels between cardiac and femoral vein specimens, with differences as high as 6.0 mg/100 ml. Other drugs whose postmortem distribution through the vascular system is more uniform than barbiturate might show significant variations between specimens drawn during life and after death.

Summary

Postmortem serum digoxin levels from any source routinely exceed antemortem values. Variation resulting from site of sampling gave a mean postmortem to antemortem ratio of 1.96 for heart, 1.63 for subclavian vein, and 1.42 for femoral vein samples.

No correlation could be made between the postmortem interval and the increase in postmortem serum values, irrespective of the site of sampling.

A combination of femoral venous serum and vitreous humor values gave the best information for determining possible antemortem digoxin toxicity.

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